

Chemical Ionization Mass Spectrometric Characteristics of Morphine Alkaloids

The ion-molecular reaction behavior of ten morphine alkaloids with several commonly used reagent gases are studied under chemical ionization mass spectrometry conditions. These studies emphasize the correlation of the structural characteristics of the 10 alkaloids with the following four mass spectrometric parameters: (i) mass shifts of the protonated ion as a result of replacing ammonia with deuterated ammonia as the reagent gas, (ii) relative tendencies of the adduct ion and the protonated ion to lose molecules of water, (iii) relative intensity ratio of the adduct ion and the protonated ion and (iv) tendency of a compound to undergo a reduction reaction.

INTRODUCTION

Reagent gas characteristics were topics of interest long before the formal adoption¹ of ion-molecule reactions as an underlying analytical mechanism in the form of chemical ionization (CI) mass spectrometry. This interest originates from the realization that ion generation and reaction are important in measuring proton affinities of selected compounds,²⁻⁴ and later, in exploring the use of various reagent gases⁵ for practical analytical purposes. Recently, deuterated reagent gases⁶⁻¹⁰ have been used for probing active hydrogens attached to a heteroatom in order to establish the class of the compound under examination.

In this report, compounds containing *more than one type of active hydrogens* are studied under CI conditions and compared with closely related compounds, in the hope that CI spectra information can be further extended for structure elucidation. For this purpose, methane, isobutane, ammonia, *d*₄-methane and *d*₃-ammonia are used for CI studies of ten morphine alkaloids. Mass spectra obtained are interpreted in terms of these compounds' relative proton affinities and the number and nature of heteroatom-bonded hydrogens present. This additional information can be used to broaden the analytical value of CI mass spectrometry by providing more structural insight.

EXPERIMENTAL

A Finnigan MAT 311A/INCOS system (San Jose, California, USA) was used for this study. The instrument was operated in the CI mode with the samples introduced through a direct evaporation rod using methane and isobutane as reagent gases and a desorption CI rod using ammonia and *d*₃-ammonia.

The mass spectrometer/data system was calibrated with perfluorokerosene-H, PCR Research Chemicals (Gainesville, Florida, USA), to establish mass assignments based on the scanning of the magnetic field as a function of time. A resolution of approximately 1000 was maintained throughout all experiments. The electron energy for the ionization of reagent gases was 150 eV. Zero potential energy and 3 kV accelerating voltage were used. The source temperature was maintained at 115 ± 5 °C when samples were evaporated and spectra registered. The reagent gas pressure was monitored by a Penning gauge between the turbomolecular pump and the source housing and was maintained at 5 × 10⁻⁵ Torr. The exact pressure at the CI box was not known but estimated as described later. Being aware of the significant effects of reagent gas pressure¹¹⁻¹³ and source temperature^{11,14-19} on ion-molecule reactions, and therefore the resulting spectra, every precaution was taken to maintain the pressure at the set conditions. No pressure change was observed on the meter while obtaining mass spectra of an entire series of compounds with a specific reagent gas. Therefore, comparison of all spectra obtained under these constant conditions is valid.²⁰ It should be noted, however, that the comparison of data obtained on different dates or with different gases should be made with extreme care, as the exact source conditions may not have been duplicated.

Reagent gases methane, isobutane and ammonia, minimum purities 99.97%, 99.5% and 99.999%, respectively, were obtained from MG Scientific Gases (Somerville, New Jersey, USA). *d*₄-Methane and *d*₃-ammonia, minimum deuterium contents 99.0% and 99.5%, respectively, were obtained from Cambridge Isotope Laboratories (Cambridge, Massachusetts, USA). The morphine alkaloids used in this study are shown in Fig. 1. The sources of these compounds are as follows: **1**, **4** and **7**, Pennick Corporation (Lyndhurst, New Jersey, USA); **2**, Alltech Associates/Applied Science Labs (Deerfield, Illinois,

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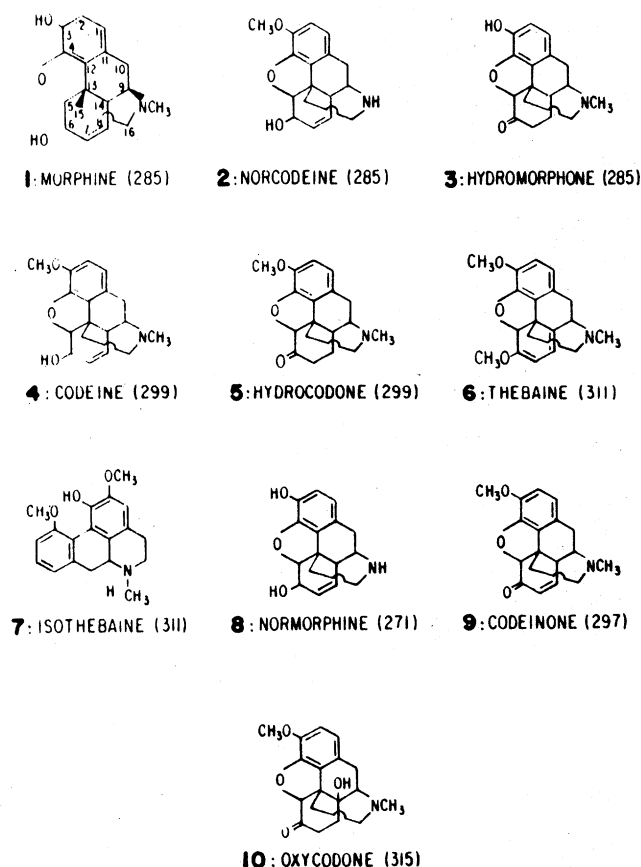


Figure 1. Structures of 10 morphine alkaloids and their numerical molecular weights.

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RESULTS AND DISCUSSION

The major advantage of CI mass spectrometry is the generation of simplified mass spectra often with single peaks related to the molecular weights of compounds under examination. These spectra are therefore very informative so far as molecular weights are concerned. By comparing spectra obtained with various reagent gases, information related to proton affinity and active hydrogens may be revealed which can provide additional structural insight and therefore increase the analytical value of the CI mass spectrometric method.

Establishment of constant source conditions

To separate solvent effects from intrinsic molecular properties, ion-molecule reactions under CI conditions have been used for the measurement of relative proton affinities of several series of compounds.^{2,3,21} The work presented here is not intended for the measure-

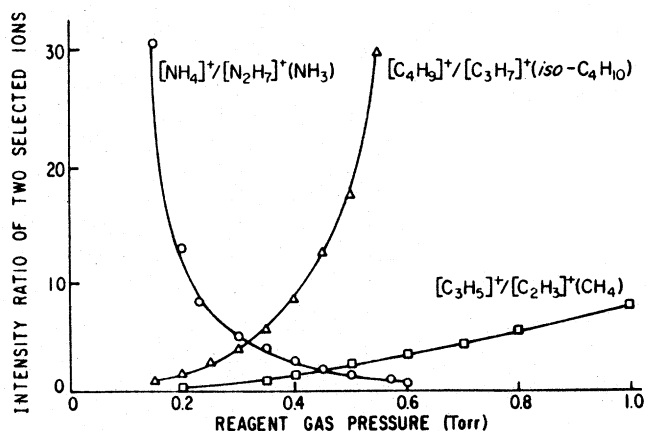


Figure 2. Relative intensities of selected ions as a function of reagent gas pressure: $[C_3H_5]^+/[C_2H_3]^+$ generated from methane at 210°C, $[C_4H_9]^+/[C_3H_7]^+$ generated from isobutane at 175°C and $[NH_4]^+/[N_2H_7]^+$ generated from ammonia at 177°C.

ment and compilation of exact gas phase basicity data.²² Rather, the intensity ratios of protonated and adduct ions observed from this series of compounds are compared and used as an indication of their relative proton affinities for compound characterization purposes. For this comparison to be meaningful, the exact reagent gas pressure and ion source temperature need not be known, but should be maintained constant. The exact pressure in the CI box was not known but was estimated as follows. Relative intensities of selected ions, as a function of pressure, were taken from the literature.^{1,11,23} Ratios of the two most characteristic ions observed in methane,¹ isobutane¹¹ and ammonia²³ were calculated and plotted in Fig. 2. The ratios of these ions observed in our experiments are 3.74 ($N=5$, std dev. = 0.029), 3.35 ($N=5$, std dev. = 0.034) and 0.0500 ($N=5$, std dev. = 0.00076), respectively. By comparing these intensity ratios with those shown in Fig. 2, the reagent gas pressure used in our experiments is estimated to be 0.62, 0.28 and 0.18 Torr for methane, isobutane and ammonia, respectively. Since the ion source geometry and other conditions may affect the intensity ratios of these ions, these values can only be treated as estimates. These pressure settings are not necessarily optimal²⁴ for obtaining CI spectra of these compounds, but are used in an attempt to maintain constant conditions throughout each series of experiments.

Characterization of compounds with identical molecular weights

From an analytical point of view, the comparison of CI spectra of compounds with the same molecular weight is the major concern. The following groups of alkaloids have identical molecular weights within each group: 1, 2, 3; 4, 5 and 6, 8. Differentiation of these compounds may be achieved based on the following parameters. (i) The number of active hydrogens in a compound determines the mass shift when ammonia is replaced by deuterated ammonia as the reagent gas.⁶⁻¹⁰ If two compounds which have an identical

Table 1. Relative intensities of protonated and adduct ions^a

		1	2	3	4	5	6	7	8	9	10
Molecular weight		285	285	285	299	299	311	311	271	297	315
Total no. of active H		2	2	1	1	0	0	1	3	0	1
No. of allylic OH		1	1	0	1	0	0	0	1	0	1
No. of phenolic OH		1	0	1	0	0	0	1	1	0	0
Reagent gas	Ion	1	2	3	4	5	6	7	8	9	10
CH ₄	[M + C ₂ H ₆] ⁺ , d	7.2	8.3	14	8.1	13	8.9	16	5.3	13	13
	[M + C ₂ H ₅ - 18] ⁺ , c	4.3	^b	—	—	—	—	—	2.7	—	1.0
	[M + H] ⁺ , b	31	35	100	28	100	100	100	28	100	100
	[M + H - 18] ⁺ , a	100	100	3.3	100	—	—	2.6	100	—	4.9
	(c + d) ^c	8.8	6.1	14	6.3	13	8.9	16	6.3	13	13
	(a + b)	100	100	100	100	100	100	100	100	100	100
NH ₃	[M + NH ₄] ⁺	81	77	100	98	100	—	—	32	98	—
	[M + H] ⁺	100	100	34	100	32	100	100	100	100	100

^a Data obtained by using isobutane as the reagent gas parallel those obtained under methane conditions and are not presented or discussed.

^b Less than 1.0% of the base peak.

^c This represents a better estimate of the adduct ion to protonated ion intensity ratio.

molecular weight differ in the number of active hydrogens, they can be differentiated based on this parameter alone. (ii) Compounds with alcoholic OH are known^{7,10} to lose a molecule of water on protonation. This parameter can be used to identify the presence of this functional group. (iii) Different compounds vary in their proton affinities²⁵ and therefore show differences in the adduct ion over protonated ion intensity ratios.

Although the three above phenomena were well known, structural elucidations based on the combined information related to adduct and dehydrated ions remained to be studied. The series of compounds studied here illustrates the possibilities concerning the use of this combined information. Detailed and quantitative interpretations are based on data presented in Table 1, and further discussed below.

Mass shift. The use of a deuterated reagent gas to provide structural insight with respect to the number of active hydrogens present has been well established.^{6,7} The mass shifts observed in *d*₃-ammonia/ammonia CI mass spectrometry are used to establish the total number of active hydrogen as shown in Table 1.

Loss of water from protonated ions. Protonation at a nitrogen site by ions derived from a reagent gas will not lead to the loss of a molecule of water. On the other hand, protonation at an oxygen site with active hydrogens attached can lead to such a fragmentation. The extent of this loss depends on whether the oxygen is present as a phenolic or an alcoholic²⁶ OH group as well as the reagent gas characteristics and source conditions. Under the methane CI conditions used throughout this series of alkaloids, an allyl hydroxyl group generates a [M + H - 18]⁺ ion of intensity three times that of the [M + H]⁺ ion, while a phenolic OH generates a [M + H]⁺ ion intensity 30 times that of the [M + H - 18]⁺ ion. The lower intensity (4.9%) of the [M + H - 18]⁺ ion observed in compound **10** is probably the consequence of an isolated hydroxyl group, steric hindrance and the rigid structural framework in which this group is situated.

By comparing the methane data with the NH₃/ND₃ data, it is possible to count the number and differentiate the nature of the active hydrogens present. For example, the mass shift information provided by the NH₃/ND₃ CI mass spectrometry indicates that both **3** and **4** have one heteroatom-bonded hydrogen; the methane CI data (Table 1) indicate that this is a hydroxyl hydrogen in compound **4**, but a phenolic hydrogen in compound **3**. Similarly, the NH₃/ND₃ data indicate that there are two heteroatom-bonded hydrogens in compounds **1** and **2**, but there is only one such hydrogen in compound **3**. The [M + H]⁺/[M + H - 18]⁺ ratios obtained by methane CI strongly suggest that the hydrogen in **3** is phenolic. Compound **1** differs from compound **2** by showing a substantial intensity (4.3%) of the [M + C₂H₅ - 18]⁺ ion. This characteristic is also observed in compound **8** which also contains both a hydroxyl and a phenolic OH group on a similar framework.

Adduct ion to protonated ion intensity ratio. It is possible to characterize further compounds based on the ratio of their adduct ion to protonated ion intensities. These ratios reflect the proton affinities of these compounds. Proton affinities, in turn, are affected by the presence of various functional groups and/or the positions of these groups. Compound **3** differs from **1** and **2** by showing a more intense adduct ion intensity with either methane or ammonia as the reagent gas (Table 1). The difference between compounds **1** and **2** are more subtle in this aspect. The two non-alcoholic hydrogens in these two compounds cause a small (8.8% v. 6.1%), but significant, difference in their adduct ion to protonated ion intensity ratios. This small difference is considered reliable because of the stable source conditions established throughout each series (reagent gas) of experiments.

The criteria used to judge the stability of the ion source condition are twofold. (i) As described in the subsection entitled 'Establishment of constant source conditions', the intensity ratio of the two ions monitored for each reagent is constant. (ii) The relative ion abundance values reported in Table 1 are precise. For

example, the abundances reported for compounds **1** (17 measurements) and **2** (7 measurements) using methane as the reagent gas, have small standard deviations shown in parentheses: 7.2 (0.44), 4.3 (0.27), 31 (0.73); 8.3 (0.50), 35 (0.77).

The small difference (2.7%) shown by the two different non-alcoholic hydrogens in compounds **1** and **2** may be overlooked in routine analysis. On the other hand, it can be easily recognized in cases where structural elucidations are emphasized and studied under controlled conditions. This difference becomes more obvious (81% v. 77%) when the more basic ammonia is used as the reagent gas.

Effect of methyl group substitution

The adduct ion to protonated ion ratio characteristics discussed above can be further illustrated by comparing data obtained from compounds **1**, **2**, **4** and **8** using the more basic ammonia as the reagent gas. Keeping other groups constant, replacing the phenolic hydrogen with a methyl group increases the relative intensity of the adduct ion which can be seen by comparing compound **1** with **4** (81–98%) and **8** to **2** (32–77%). Similarly, replacing an amine hydrogen with a methyl group also causes an increase in the adduct ion intensity as observed when comparing compound **8** with **1** (32–81%) and **2** with **4** (77–98%). These data clearly indicate the reduced substitution effect of the second methyl group whether it replaces an alcoholic or an amine hydrogen.

Reduction under CI conditions

During the course of this study, compound **9** is observed to undergo reduction similar to some keto acids reported in the literature.²⁷ Our limited data cannot reveal the mechanistic aspect of this reaction; we simply wish to point out the characteristics of this compound. This phenomenon should however be recognized when qualitative and quantitative analyses of unsaturated compounds are addressed.

In summary, the number of heteroatom-bonded hydrogens in a compound is revealed by the mass shift as a result of replacing NH_3 with ND_3 as the reagent gas. The adduct ion to the protonated ion intensity ratio and the tendencies of these ions to lose molecules of water provide additional information related to the nature of these hydrogens, and the ion-molecule reaction characteristics of compounds under examination. It should be noted, however, that without knowing the exact nature of the intramolecular interaction of these functional groups, interpretation beyond comparison purposes should be made with extreme caution.

Acknowledgements

This work was partially supported by Grant No. I-368-81 from BARD (The United States–Israel Binational Agricultural Research and Development Fund). The authors wish to thank P. G. Vincent, E. J. Cone, D. Lavie and P. Mahlberg for providing some of the alkaloids used in this study.

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